Abridged from the

Dermatology Foundation

Progress in Dermatology

Editor: Alan N. Moshell, M.D.

Skin–Nervous System Interactions

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Components of the cutaneous nervous system interact with multiple types of cells in the skin to mediate actions important in skin inflammation and wound healing [1–5]. Over 100 years ago it was observed that antidromic stimulation of the sensory nerves emanating from dorsal root ganglia produced vasodilation and apparent local inflammation of the skin (for review see [6]). This observation, repeated many times since, led to the understanding that the cutaneous sensory nervous system (CSNS) not only relays sensory information from the skin to the central nervous system (CNS), but also plays an effector role in the skin's inflammatory response [7–9].

Sensory neurons express at least 17 different neuropeptides [6]. Several of these, substance P (SP) [6,10,11], calcitonin gene-related peptide (CGRP) [12–14], substance K (SK) [10,15,16], and vasoactive intestinal polypeptide [17–20], have activities or cellular localizations that suggest involvement in tissue repair. SP is the prototype of neuropeptides released from sensory C-fibers in the skin [21]. The activities of SP require not only secretion but also the expression of the SP receptor (SPR) on local target cells and expression of tissue proteases that degrade neuropeptides, such as neutral endopeptidase (NEP).

Another important facet of nervous system–cutaneous interactions are neurotrophic growth factors that mediate cutaneous reinnervation, e.g., nerve growth factor (NGF) [22,23], brain-derived nerve growth factor [24,25], neurotrophin-3 [26], and human neurotrophin-5 [27]. NGF is produced by the neural Schwann cells and by the regenerating epidermis itself [28,29].

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Abbreviations: CGRP, calcitonin gene-related peptide; CNS, central nervous system; CSNS, cutaneous sensory nervous system; NEP, neutral endopeptidase; SK, substance K; SP, substance P; SPR, substance P receptor.

BIOLOGIC ACTIVITIES OF SP AND SPR

SP Biochemistry and Tissue Distribution SP, an 11–amino acid peptide that is a member of the tachykinin family of neuropeptides, is present in many areas of the nervous system but is one of a few neuropeptides restricted to cells of neural crest origin [30,31]. It is located in nerve endings throughout the body, but especially in areas of immunologic importance such as the skin, gastrointestinal tract, respiratory tract, and eye [31]. In the peripheral nervous system, SP occurs in a subpopulation of primary afferent neurons including A-δ pain fibers and C-fibers, which transmit impulses initiated by noxious stimuli, and in nerve plexuses of the gastrointestinal tract. The role of SP as a neurotransmitter is supported by its presence in synaptosomal fractions and large granular vesicles in nerve endings of synapses within dorsal horn cells [32].

SP is synthesized in the dorsal root ganglia, from which it migrates centrally to the dorsal horn of the spinal cord and peripherally to nerve terminals of sensory neurons [21]. A single gene has been identified that can be differentially processed into two different SP precursor mRNAs, α- and β-preprotachykinin [33,34]. The first contains the sequence of SP only whereas the second contains the sequences of SP as well as a second tachykinin-peptide, SK [33,35]. SP and SK share a common spectrum of biologic activities [10,36,37], but levels of the two precursor mRNAs vary between tissues [33,38], the CNS producing more α-preprotachykinin and the peripheral nervous system (PNS) producing more β-preprotachykinin [34]. SP is degraded by a specific thiorphan-sensitive enkephalinase, CD10, also known as NEP [39–41].

SP Receptor Biochemistry The amino acid sequence of the human and rat SPR neurokinin (NK_1), has been deduced from molecular cloning data [42–44]. The receptor protein is composed of 407 residues with seven putative membrane-spanning domains and shares sequence homology with the SK receptor (NK_2) in its transmembrane regions and in its first and second cytoplasmic loops [45,46]. The SPR mRNA is expressed by neurons and glia in the

0022-202X/96/$10.50

Table I. *In Vitro* Effects of the Neuropeptide Substance P on the Immune System

Effect	Specifics
Cellular proliferation	• Enhances T-cell proliferation (much greater in Peyer's patches than in spleen) [98]
Ig production	• Increase IgA and IgM [102]
	• Increase heavy-chain mRNA [102]
Cytokine production/secretion	• Enhances IL-2 synthesis and secretion by peripheral blood monocyte cells and HUT 78 cells [105]
	• Enhances IL-2 receptor expression on Jurkat-SP-R cells [104]
	• Enhances IL-1, IL-6, TNFα, and interferon γ production by monocytes [103]
	• Enhances expression and secretion of IL-1α, IL-1ra, and granulocyte/macrophage colony-stimulating factor by keratinocytes [57]
	• Enhances TNFα secretion by mast cells [56]
Phagocytosis	• Enhances macrophage and polymorphonuclear–leukocyte activity [108,185,186]
Mediator release	• Enhances histamine, prostaglandin, and leukotriene release from mast cells [106]
Cell homing, chemotaxis	• Enhances human monocyte chemotaxis [186,187]
	• Alters CD2 and CD45 levels on Jurkat-SP-R cells [104,188]

CNS, neurons of the myenteric plexus, smooth muscle cells, acinar cells, endothelial cells, fibroblasts, various types of circulating immune cells, and skin [42,47–50]. Sites of expression agree well with earlier autoradiographic localizations [51–53]. SP, with a kD of 0.5–1.0 nM, is the most potent ligand for the NK_1 receptor [54]. Activation of the NK_1 receptor results in inositol phospholipid hydrolysis and the transient appearance of inositol 1,4,5 triphosphate, the release of Ca^{++} from internal stores, and an oscillatory chloride current [55]. We have identified SPR in the skin on keratinocytes and mast cells [56,57], whereas other recent studies have demonstrated SPR on endothelial cells [58,59].

Actions of SP In the CNS, SP is thought to act in the cortex, hypothalamus, and lower brainstem [60–67]. SP in the PNS has numerous actions including contraction of the smooth muscle layers in the gastrointestinal tract [68,69]; production of edema and bronchoconstriction in the respiratory tract [70–72]; enhancement of nasal secretion [70,73]; stimulation of tracheal, epithelial, and glandular secretion of glycoprotein-rich fluid [74]; calcium-dependent secretion by the parotid and submandibular glands [75]; and modulation of both exocrine and endocrine pancreatic function [76–79]. Blood and tissue concentrations [80–82] of SP suggest that the peptide is available locally at functionally relevant concentrations. The peripheral actions of SP possibly involved in cutaneous neuroinflammation fall into three main categories: 1) vasodilation and increased vascular permeability, 2) local inflammation and effects on the immune system, and 3) promotion of cellular proliferation.

Vasodilation and Increased Vascular Permeability Increased blood flow and vascular permeability are effects well established in several animal species and humans [83–85]. Vasodilation is due to direct actions of SP on vascular smooth muscle [85,86] and enhanced production of nitric oxide by the endothelium [87]. SP is also the mediator of antidromic vasodilation, as shown by its presence in unmyelinated primary sensory fibers in the skin [86,88], its release from peripheral endings of sensory neurons during antidromic stimulation [89,90], and its vasodilatory effects after venous infusion [91]. In addition, SP can initiate microvascular permeability and protein extavasation after tissue injury [31,91,92]. Intradermal injection of SP in humans produces a wheal caused by increased vascular permeability followed by a flare reaction caused by reflex neurogenic vasodilation [93–95]. The reflex nature of the flare is demonstrated by its inhibition by local anesthetics; the wheal response remains unaffected [96,97].

Local Inflammation and Specific Effects on the Immune System SP has been shown to have stimulatory effects on the immune system both *in vitro* and *in vivo* [98–100] **(Table I)**. Initially, SP was found in high concentrations in inflamed tissues such as the joints in experimental arthritis in the rat [101]. Later, injections of SP into normal joints produced inflammation that mimicked adjuvant-induced arthritis [101].

Nanomolar concentrations of SP enhance proliferation of both human and murine peripheral-blood T lymphocytes [98,99]. [^{3}H]-thymidine and [^{3}H]-leucine uptake by human T lymphocytes is stimulated up to 60–70% by both SP and a C-terminal fragment of SP, SP_{4-11}, both in the presence and absence of other mitogens [98]. SP also increases, by up to 300%, the synthesis of immunoglobulins from mixed lymphocyte cultures from the spleen, lymph nodes, and Peyer's patches, the major effect being on IgA [99]. More recently, Pascual *et al*, using an IgM- and IgA-producing B-lymphoma cell line, showed that SP can synergize with lipopolysaccharide to increase the production of these two immunoglobulins [102]. Continuous infusion of SP *via* miniosmotic pumps stimulated proliferation of lymphocytes isolated from the spleen and Peyer's patches in mice, and immunoglobulin synthesis, particularly IgA, was also increased [100]. SP has effects on cytokine regulation including enhanced production of cytokines such as interleukin 1 (IL-1), IL-6, tumor necrosis factor α (TNFα), and interferon-γ by monocytes [103], increase in IL-2 receptors in a Jurkat T-cell line expressing a transfected SPR cDNA [104], and augmented IL-2 synthesis in peripheral blood monocyte cells and HUT 78 cells [105].

SP can also modulate acute hypersensitivity responses. At micromolar concentrations SP evokes the release of histamine from rat serosal or connective-tissue–type mast cells [106]. Unlike other basic peptides, which stimulate mast cells nonspecifically, SP action is cell specific and includes stimulation of the generation of unstored mediators such as leukotrienes [107,108]. The mast cell responses elicited by SP were distinguished from those dependent on IgE by the apparent lack of a requirement for extracellular calcium [106].

We have recently demonstrated [56] that mast cell TNFα mRNA is selectively up-regulated by SP in a concentration-dependent manner. SP increased secreted TNFα from both cloned murine CFTL 12 mast cells and freshly isolated peritoneal mast cells. The addition of SP to cultured murine and normal human foreskin keratinocytes resulted in concentration-dependent increases in mRNA for IL-1α, IL-1β, and IL-1ra that were maximal after 3 h of exposure ([57], unpublished observations), but no effects on keratinocyte TNFα or IL-8 were found. It is interesting that SP_{1-9}, but not the C-terminal fragments SP_{4-11} or SP_{7-11}, are capable of inducing production of some cytokines by keratinocytes. More recently, we observed that SP can also specifically activate normal human microvascular endothelial cells to produce the neutrophil activating cytokine IL-8.¶

Promotion of Cell Proliferation Neuropeptides, especially SP, have also been implicated in the modulation of inflammation and wound healing by the ability to promote proliferation of various types of target cells. SP has been shown to act through the

¶ Kramp J, Brown J, Cook P, Russell B, Lawley T, Armstrong C, Ansel J: Neuropeptide induction of human microvascular endothelial cell interleukin 8 (abstr). *J Invest Dermatol* 104:586, 1995.

SPR to stimulate proliferation of cultured keratinocytes [11]. Additionally, SP and SK can stimulate proliferation of arterial smooth muscle cells and therefore may assist in angiogenesis during healing [10,16,109]; SP has also been shown to stimulate neovascularization *in vivo* [110]. SP and SK stimulate the proliferation of both human skin fibroblasts [10] and cultured endothelial cells [110], suggesting that SP released from the skin in association with tissue injury may not only enhance vasodilation and the inflammatory response, but may stimulate proliferation of cutaneous epithelial, vascular, and connective tissue.

ROLE OF NEP IN REGULATING NEUROPEPTIDE ACTIVITY

NEP Biochemistry and Tissue Distribution The biologic actions of neurotransmitters such as acetylcholine are terminated by enzymatic degradation, by re-uptake into nerve endings, and/or by diffusion away from the target cell [111,112]. Accumulating evidence suggests that neuropeptides are degraded and inactivated by cell surface peptidases [113]. The best-studied cell surface peptidase, neutral endopeptidase (EC 3.4.24.11, NEP), also known as enkephalinase, common acute lymphoblastic leukemia antigen, or CD 10, is the major tachykinin-degrading enzyme. NEP was discovered in the brush border of the kidney in 1968 as an enzyme that hydrolyzed the β chain of insulin [114] but was not obtained in pure form until 1974 [115,116] and has also been isolated from other tissues. Interest in NEP was rekindled in 1978 when NEP was shown to degrade enkephalins in the brain [117]. NEP degrades tachykinins, opioid peptides, GRP-10, somatostatin-14, neurotensin, cholecystokinin (CCK), gastrin, vasoactive intestinal polypeptide, CGRP, and others [118–120]. Although tachykinins and enkephalins are the best substrates, the proximity of peptidergic neurons to cells that express NEP also determines whether a neuropeptide can be a relevant physiologic substrate for NEP. The consensus is that NEP in the nervous system is found in neurons rather than glial cells. In the dorsal horn of the spinal cord and the trigeminal nucleus, staining for NEP, SP, and enkephalins is superimposable.

The search for a physiologic role of NEP has been facilitated by use of the specific inhibitors phosphoramidon and thiorphan. The release of both enkephalins and SP from brain slices *in vitro* is enhanced in the presence of NEP inhibitors [121,122], and these inhibitors relieve pain [122–124], which is consistent with their effects on endogenous opioid peptide release and stability. In the airways, inhibitors of NEP potentiate effects of SP on tracheal secretion [74], bronchomotor tone [125], and cough [126]. In addition, inhibition of NEP has been shown to potentiate tracheal neurogenic inflammation [127]. The mechanism by which NEP modulates the effects of neuropeptides is widespread and phylogenetically ancient. For example, phosphoramidon stimulates the growth of small-cell lung carcinoma cells by preventing the inactivation of endogenous GRP (an autocrine growth factor) by NEP [128]. An NEP-like enzyme is also found in hemocytes of the mollusc *Mytilus edulis*, and inhibition of NEP activity decreases by five orders of magnitude the amount of Met-enkephalin required to activate these cells [129].

Although regulation of NEP activity and expression has received little attention, changes in its activity have profound effects on the cellular response to neuropeptides. NEP activity in the trachea is reduced by up to 50% by infection [130], and NEP is down-regulated by intestinal inflammation; as a result, the degradation of SP by inflamed tissue is markedly reduced [131]. The ability of glucocorticoids to stimulate expression of NEP by transformed tracheal epithelial cells [132] and to down-regulate SPR [133] may partly explain their anti-inflammatory properties.

CUTANEOUS NGF

NGF Biochemistry and Tissue Distribution NGF, a 118–amino acid, ~130-kD protein isolated more than 40 years ago as an agent stimulating growth and neurite development in embryonic sensory cells [134], is the best-characterized member of the neurotrophin family [135]. NGF is composed of three subunits, α, β, and γ, but biologic activity appears to reside in the 26-kD β subunit (2.5S NGF) [136].

In the mature animal, NGF is expressed in the CNS and peripheral tissues [137,138] including keratinocytes of mouse and human epidermis [28,29,139]. NGF mRNA in keratinocytes is increased by ultraviolet radiation and phorbol esters, and reduced by hydrocortisone [28,29]. Anti-NGF monoclonal antibody stained only basal keratinocytes obtained from growing colonies; cells from confluent cultures and fully differentiated cells showed no evidence of NGF staining [29], suggesting a role for NGF production by the regenerative epidermis in wound healing.

NGF Receptor One NGF receptor (NGFR) protein is a more abundant low–molecular-weight protein of ~75 kD, $p75^{NGF\text{-}R}$, that binds NGF with low affinity, and the second is a ~140-kD protein that binds NGF with high affinity [140]. Both are required for high-affinity binding and a normal cellular response. The transmembrane protein $p75^{NGF\text{-}R}$ appears to serve as a common subunit for different neurotrophin receptors, whereas the specificity of the receptor is conferred by the second subunit [141]. The higher–molecular-weight subunit is now known to be the proto-oncogene, *trk*, a member of the tyrosine kinase receptor family [142,143].

General Biologic Actions of NGF NGF has been shown to be both neurotrophic and neurotactic in the CNS. The outgrowth of processes from ganglion neurons is dependent on NGF [144], and NGF is also crucial for neuronal survival and function. Exposure of fetal rats to antibodies to NGF causes the ablation of an entire population of sensory neurons including those containing SP [145,146]. NGF is taken up by sensory neurons and transported in retrograde fashion to the cell body [147]. Deprivation of NGF results in reduced levels of SP in dorsal root ganglia [148].

Effects of Wounding on Skin Innervation Innervation of skin is altered by wounding [149–153]. In rat and guinea pig skin, SP-containing nerve fibers disappear from wounded skin within 1–2 d [152,153] and regenerating fibers begin to return after 3 d, increasing gradually for ~2 weeks after wounding and resulting in hyperinnervation. The new fibers exhibit extensive sprouting and form an unusually dense network just beneath the regrowing epidermis and then decrease in number over several weeks, following the course of healing and suggesting a functional link between regeneration of the CSNS and the integrity of the skin.

Role of NGF in Reinnervation of Wounded Skin After injury to a cutaneous nerve, denervated skin is reinnervated by two mechanisms, axonal regeneration (i.e., the regrowth of injured axons) and collateral reinnervation (i.e., the sprouting of undamaged axons in adjacent tissues into the denervated area) [154]. The latter has been shown to be completely dependent on NGF, because reinnervation does not occur after depletion of NGF [155]. Furthermore, collateral reinnervation is blocked by passive or active immunization against NGF [23,156]. Diamond *et al* went on to show that intradermal injection of NGF produced collateral sprouting in normal, fully innervated skin, increased the rate of sprouting in denervated skin, and restored the rate of sprouting in anti–NGF-treated denervated skin [23]. Finally, there appears to be an increase in synthesis of NGF within denervated tissue [23,157]. As mentioned above, keratinocytes express and synthesize NGF and may be the source of the endogenous growth factor utilized in sprouting. This is especially likely because a) reinnervating nerves enter the wound from the edge along with the epithelial tongue [152] and b) there is an unusually dense arborization of nerves just beneath the rapidly growing keratinocyte layer, which contains keratinocytes known to express the highest level of NGF [29]. It is possible, however, that NGF from Schwann cells, fibroblasts, and smooth muscle cells may also be involved.

NGF may also play a significant role in axonal regeneration after injury [158]. This hypothesis is based upon several observations: a) dorsal root ganglia have high-affinity NGF receptors [140]; b)

Schwann cells make NGF [159]; c) NGF and NGFR levels increase in degenerating nerve pathways and then become down-regulated when regenerating axons arrive [160]; and d) when peripheral nerves are cut and begin to degenerate, their Schwann cells start to express low-affinity NGFR and this expression disappears when the regenerating axon arrives [158,160]. It is thought that NGF is bound to the NGFR on Schwann cells and is then transferred to high-affinity NGFR on the regenerating axons because of the differences in binding affinities of the two types of receptor. The role of NGF in axonal regeneration has been questioned recently by evidence that nerve regeneration is unaffected by treatment with anti-NGF antibodies [161].

Proinflammatory and Proliferative Actions of NGF Paracrine actions of NGF may also contribute to the maintainance of the integrity of skin. NGF causes proliferation and release of histamine from mast cells [162], promotes differentiation of specific granulocytes, enhances T-lymphocyte–dependent antibody synthesis, and induces growth and differentiation of human B lymphocytes [163,164]. Furthermore, NGF may modulate melanocyte gene expression, is chemotactic for melanocytes, and can enhance melanocyte dendricity [139].

ROLE OF THE NEUROLOGIC SYSTEM IN WOUND HEALING

Effects of Denervation on Wound Repair It is also an old observation that chronic wounds and ulcers occur in skin with absent or dysfunctional innervation, an event often attributed to pressure, ischemia, infection, or incidental trauma related to a lack of sensation. Once such wounds were formed, failure to heal was attributed to difficulty with hygiene, protection of the wound from additional trauma, worsening of the underlying disease, poor nutrition, and susceptibility to infection. As early as 1927 it was noted that damage to the PNS is capable of altering growth and repair of skin, leading ultimately to ulceration and necrosis of the affected area [165]. Such observations led to the hypothesis that cutaneous innervation provides "trophic" support to skin integrity. Interest in this phenomenon has focused primarily on the role of sensory nerves, because it was noted that the flare response, a reflex of the CSNS [166,167], is absent in denervated skin that develops chronic wounds. Additional information has subsequently implicated the CSNS as a major contributor to skin integrity. For example, diseases and injuries that produce CSNS deficits (e.g., diabetes [168], herpes zoster [169], and spinal cord injury [170,171]) are often characterized by poor wound healing.

Denervation of rabbit skin by cutting of the nerves impairs the healing of incisional wounds [172]. Scars in denervated skin showed decreased tensile strength, hydroxyproline content, and protein composition compared to those in control skin. Near total sensory denervation can also be accomplished by treatment with high doses of capsaicin [173]. Kjartansson *et al* studied the effects of capsaicin-induced sensory denervation on the survival of tissue in experimental critical skin flaps [174]. Ten days after surgery, only 32% as much tissue remained in flaps of denervated skin as compared to flaps of innervated skin. Severe corneal lesions with opacity, edema, and ulceration can be produced in neonatal mice and rats by ablation of sensory neurons with capsaicin [175]. Similar results can be obtained by cutting the trigeminal nerve [176]. In a related experiment, depletion of neuropeptides in rabbit corneas by topical or retrobulbar injection of capsaicin caused a delay in corneal wound healing [177]. Such treatments reduce the migration rate of epithelial cells surrounding an n-heptanol–induced epithelial wound. These findings support the hypothesis that the trophic effects of sensory nerves on corneal or epidermal epithelium are mediated by neuropeptides in the sensory nerve endings.

Changes in Tissue Neuropeptide Levels After Wounding The few studies that have been conducted regarding neuropeptide levels during wound healing are difficult to compare due to vastly differing experimental models and neuropeptides of interest. Jonsson *et al* showed an immediate increase in SP immunoreactivity in dog paw lymph after scalding injury, indicating release of SP during wounding [178]. Grönblad *et al* observed many more SP and CGRP nerve fibers in healing rabbit medial collateral ligaments as compared to normal ligaments, suggesting involvement of these nerves in healing [179]. Senapati *et al*, however, observed significant depletion of SP, CGRP, and somatostatin in rat skin [180] within 1 d of wounding that persisted for 14 d, consistent with release of neuropeptides at the wound site but also possibly explained by decreased synthesis and transport to the nerve terminals. These data, which support the ideas of an increase in neuropeptidergic tone at the wound site, are further substantiated by studies in which neuropeptide replacement enhanced wound repair. In a preliminary study, Spevak *et al* found that wounds in rat skin healed faster when treated with SP [181]. English *et al* demonstrated a trophic action of the CSNS on wound healing by showing that inclusion of touch domes in skin grafts enhanced the return of neural function in the graft and, most importantly, the quality and survivability of the graft [182,183]. (Touch domes are specialized areas of skin that are richly innervated and are known to provide tropic or trophic factors for regenerating nerves [184]).

CONCLUSIONS

There is increasing evidence that the nervous system can modulate tissue inflammatory, proliferative, and reparative processes. The role of the CSNS and neuropeptides such as SP in inflammation and tissue repair is determined not only by the synthesis and secretion of neuropeptides such as SP, but also by availability of and interaction with its receptor on target cells and by the rate of SP degradation by tissue peptidases such as NEP. Furthermore, the rate at which reinnervation of wounds occurs may have dramatic effects on the time course and quality of tissue repair, thus pointing toward a crucial role for neurotropic and neurotrophic factors like NGF.

Our current understanding of the different components of this complex system suggests that defects in skin integrity and wound healing in patients with neurologic sensory deficits may be caused by abnormalities in growth and function of the CSNS. In view of the scale of the clinical problem represented by chronic wounds, increasing our knowledge of the interaction between the CSNS and wound healing has the potential for making a significant impact on therapy.

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